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(54) Title: METHOD TO INCREASE STRESS TOLERANCE IN PLANTS

(57) Abstract: A method is presented for selecting and isolating nucleic acids capable of conferring tolerance or resistance to environmental stress conditions in plants or yeast. Furthermore, nucleic acids, the proteins they encode and their use for the production of plants or yeast with enhanced environmental stress resistance is disclosed.

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# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/EP2004/050513

**A. CLASSIFICATION OF SUBJECT MATTER**  
IPC 7 C12N15/82 C12N15/81 C07K14/415

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)  
IPC 7 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, WPI Data, PAJ

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 02/052012 A (CROPDESIGN N V ; SERRANO SALOM RAMON (ES); ROS PALAU ROQUE (ES); KANHO) 4 July 2002 (2002-07-04) the whole document -----	22-26
A	JEONG M-J ET AL: "Isolation and characterization of the gene encoding glyceraldehyde-3-phosphate dehydrogenase" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, ACADEMIC PRESS INC. ORLANDO, FL, US, vol. 278, no. 1, 11 November 2000 (2000-11-11), pages 192-196, XP002255015 ISSN: 0006-291X the whole document ----- -/-	1-26

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

\* Special categories of cited documents :

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

- \*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- \*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- \*&\* document member of the same patent family

Date of the actual completion of the international search  25 November 2004	Date of mailing of the international search report  01.03.2005
Name and mailing address of the ISA  European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl. Fax: (+31-70) 340-3016	Authorized officer  Hermann, P

## INTERNATIONAL SEARCH REPORT

International Application No  
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C(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KIM J C ET AL: "A novel cold-inducible zinc finger protein from soybean, SCOF-1, enhances cold tolerance in transgenic plants" PLANT JOURNAL, BLACKWELL SCIENTIFIC PUBLICATIONS, OXFORD, GB, vol. 25, no. 3, February 2001 (2001-02), pages 247-259, XP002973197 ISSN: 0960-7412 the whole document -----	1-26
A	RODRIGUEZ-VARGAS SONIA ET AL: "Gene expression analysis of cold and freeze stress in baker's yeast" APPLIED AND ENVIRONMENTAL MICROBIOLOGY, vol. 68, no. 6, June 2002 (2002-06), pages 3024-3030, XP002304505 ISSN: 0099-2240 the whole document -----	1-26
A	HOWARD TIFFANI L ET AL: "CHMP1 functions as a member of a newly defined family of vesicle trafficking proteins" JOURNAL OF CELL SCIENCE, vol. 114, no. 13, July 2001 (2001-07), pages 2395-2404, XP002304506 ISSN: 0021-9533 the whole document -----	1-26

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/EP2004/050513

### Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
  
2.  Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
  
3.  Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

### Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1.  As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
  
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
  
3.  As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
  
4.  No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the Invention first mentioned in the claims; it is covered by claims Nos.:

22-26 (in full) and 1-21 (in part)

#### Remark on Protest

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 22-26 (in full)

Invention 1 relates to a screening method for identifying nucleic acids and proteins encoded by said nucleic acids, capable of modifying tolerance or resistance to cold stress conditions in plants or yeast.

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2. claims: 1-21 (in part)

Invention 2 relates to the subject-matter of claims 1-21 insofar as restricted to the proteins designed CRY01-3, having the sequences given in SEQ. ID. 2, 4, 6 and/or encoded by the nucleic acid sequences given in SEQ. ID. 1, 3, 5.

Invention 2 includes i ) a method for increasing abiotic stress tolerance in plants, said resistance being linked to a modulated expression of proteins named CRY01, CRY02 and CRY03 (i.e. SEQ. ID. 2, 4, 6 and sequences presenting various degree of homology with the said sequences as indicated in part b) of claim 2), said method comprising the steps of introducing genetic modifications in said plants; ii) a method of producing a transgenic plant; iii) the plant, plant part or plant cell obtained by said methods; iv) the use of nucleic acid encoding the said protein for modifying stress tolerance in plant or yeast; v) the said nucleic acids and the said proteins corresponding respectively to SEQ. ID. 1, 3, 5 and SEQ. ID. 2, 4, 6; and vi) the genetic construct comprising the said nucleic acids.

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3. claims: 1-21 (in part)

Invention 3 relates to identical subject-matters as invention 2 when the proteins concerned are those comprising a SEC14 domain and exhibiting lipid transfer activity, said protein includes therefore CRY04 corresponding to SEQ. ID. 8 and encoded by SEQ. ID. 7.

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4. claims: 1-21(in part)

Invention 4 relates to identical subject-matters as invention 2 when the proteins concerned are those comprising a Ring finger, a serine rich domain and an acid domain which comprise the signature given in part (iii) of claim 1, said protein includes therefore CRY05 corresponding to SEQ. ID. 10 and encoded by SEQ. ID. 9.

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**INTERNATIONAL SEARCH REPORT**

Information on patent family members

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Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO 02052012	A 04-07-2002	CA 2432380	A1	04-07-2002
		WO 02052012	A2	04-07-2002
		EP 1343875	A2	17-09-2003
		JP 2004524015	T	12-08-2004
		US 2004111769	A1	10-06-2004